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14. ABSTRACT

This work is focused on developing a breast cancer biomarker utilizing magnetic resonance spectroscopy/imaging and hyperpolarization. I have initiated and completed most of the elements in the first part of the project which consist of developing the equipment and the biomarker agent. Task 2-4 required the completion of Task 1. The most significant achievement is the completion of the required equipment and initial testing. We have developed a fully functional parahydrogen induced polarization (PHIP) instrument and verified the functionality with a PHIP agent. Task 1a the development of a fixed field magnet was abandoned due to construction difficulties and better alternative solution to allowing us to generate a stable homogeneous magnetic field with a current controlled solenoid. This project was extended 6 months due to delivery problems for the required transgenic mice. We are on schedule to begin and complete Task 2-4, the in vivo an in vitro proof of concept testing of the accumulation of 15N-labeled choline analog in breast cancer cells by hyperpolarized 15N.

15. SUBJECT TERMS

Hyperpolarization, 15-nitrogen, NMR, MRI, PHIP, parahydrogen induced polarization, breast cancer, 15N, magnetic resonance imaging, nuclear magnetic resonance spectroscopy

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Introduction

We are investigating developing a new investigative method based on a magnetic resonance spectroscopy method which is concluding that elevated levels of choline serve as a marker for malignant tumors which need to be treated. The problem with current 1H spectroscopic methods is the vastly reduced signal obtained as a result of the low concentration of choline and a thermal equilibrium distribution dictated by Boltzmann distribution. Hyperpolarization is a technique being used to enhance magnetic resonance signal with a 100,000 fold increase in signal for 15N. Such immense signal allows for trace detection of 15N compounds such as choline as well as possible metabolites formed within cells.

Two model systems will be tested. Human cancer cell lines tested *in vitro* and mouse focal breast tumor models *in vivo*. These two models will test the concepts of choline metabolism detection and localization of focal tumors. An effective mouse model experiment would demonstrate an increased choline uptake of cancer cells due to an increase choline transporter activity in malignant cancer cell lines and possible metabolic products with different distinguishable resonances. In essence, we will be evaluating a method which will produce a "metabolic fingerprint" which should be specific to lesion types, benign or malignant. We envision that such highly sensitive methods will be able to detect malignant lesions.

Body

Task 1. Produce a high polarization (>10%) of choline by parahydrogen induced polarization by improving instrumentation and pulse sequences.

1a. Construct fixed field magnet for polarization equipment.

Our original concept was to utilize neodymium magnetics in a Halbach array to produce a stable highly homogenous field by use sixteen pole locations and to have a magnetic which was approximately 10 inches in diameter and 12 inches high. This would have required affixing six 2-inch length magnets to produce the 12 inch length. This ended up being very difficult to maintain the magnetic field alignment during the drying of the adhesive. We decided to replace this idea with a wrapped solenoid with a well-controlled current regulation.

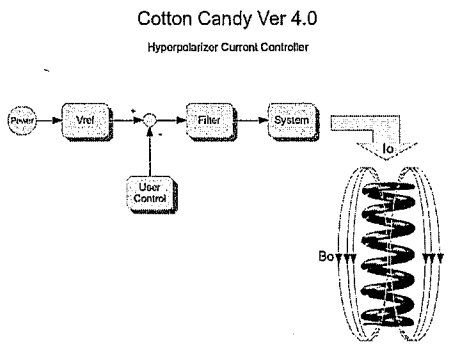

Figure 1: Flow diagram of the current regulator for producing a stable current resulting in a stable magnetic field

Cotton Candy is a low noise high stability Bo coil current controller. This circuit is based on a high stability voltage reference (20 ppm/1K hr) and a floating current source, controlled by the user through a voltage divider. Cotton candy is capable of sourcing 1.5A at 5V supply with 15ppm noise, but larger currents can be achieved at high voltages. We are currently, using the circuit to supply 800 mA to produce a 1.6mT magnetic field.

1b. Use 15N enriched choline to determine the intra-proton-proton and intra-proton-15-nitrogen couplings to enhance polarization transfers for the parahydrogen to 15-nitrogen.

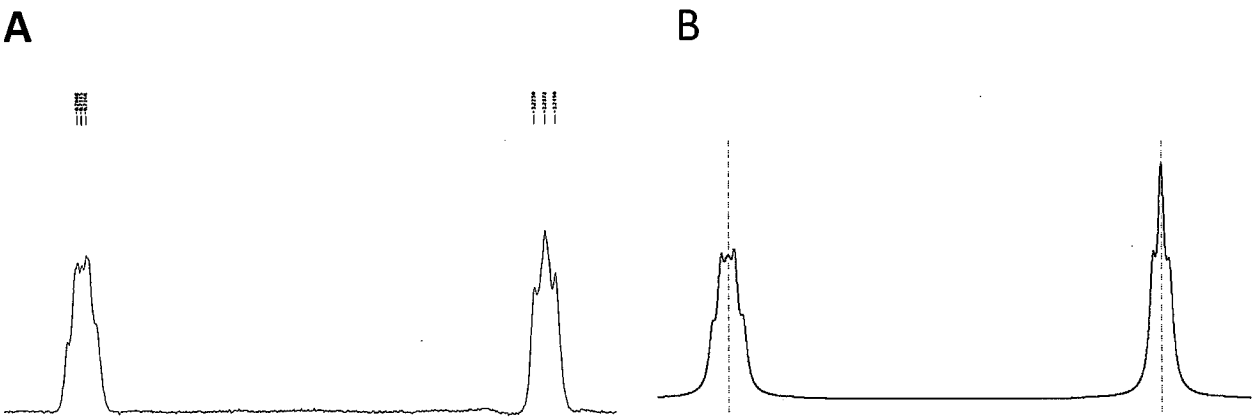

Figure 2: Choline 1H spectra from 15N-Choline. A is the actual acquired spectra and B is the modeled data with the scalar coupling constants of JH1N=6.49, JH2N=0 and JH1H2=4Hz.

We acquired the proton spectrum of a 15N labeled choline molecule and determine the scalar coupling constants by the interactions resulting in resonance splitting. The chemical shifts splitting were measured to determine J-couplings by examination of the spectrum. To confirm our values we model the interaction to derive the expected theoretical spectral which matched our experimental data.

1c. Develop working polarization transfer sequence for a parahydrogen polarizer.
These values have been determined and the transfer sequence has been created in our own proprietary software based on a prior publication on polarization transfer.[1]

1d. Determine optimal conditions for hydrogenation of neurine to choline to insure near 100% conversion to choline to reduce toxicity.

We have tested and developed the hydrogenation reaction of neurine to form the choline analog utilizing natural abundance neurine. In Figure 2 the peaks in the 5-7 ppm range are attributed to protons attached to the double bond carbons. These peaks are shifted in the hydrogenation when the double bond is hydrogenated to form a single bond. The new peaks at 3.1 and 3.3 ppm are from the hydrogens on the carbons after hydrogenation and larger peak at 2.2 ppm is mainly due to the methyl protons on the nitrogen atom. The spectra show that we can achieve full hydrogenation at 4 seconds of reaction time with the correct number of protons added; this is determined by the intensity of the peaks.

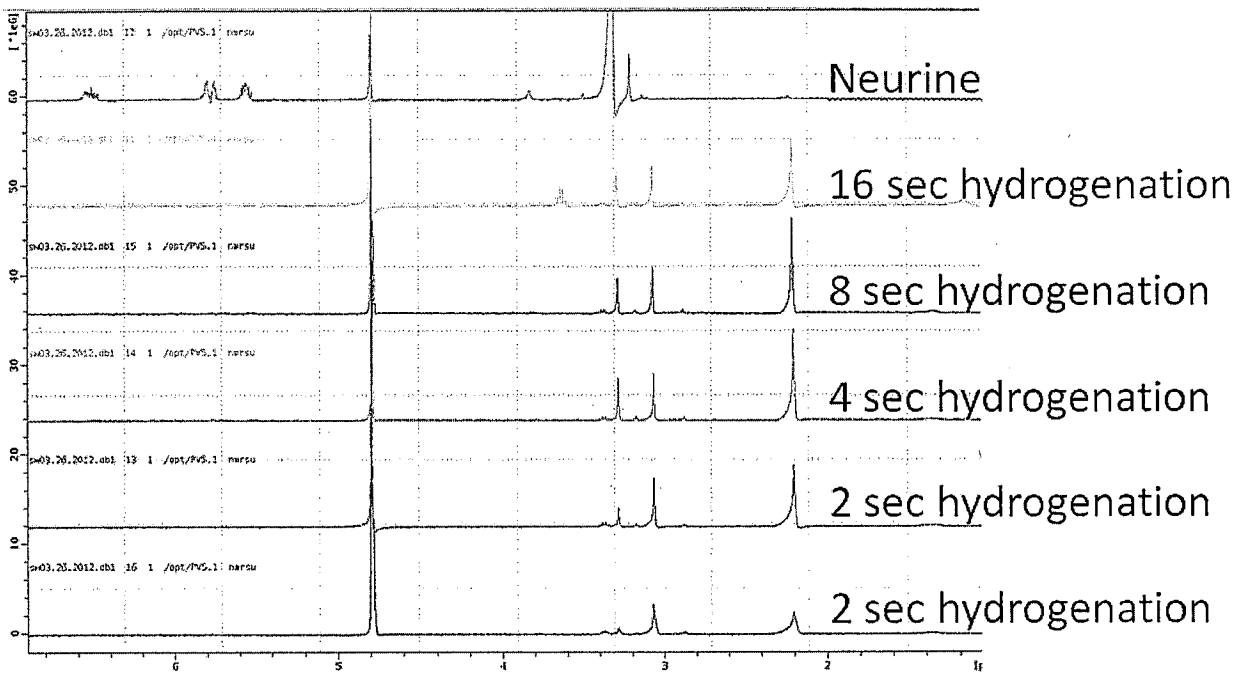

Figure 3: 1H proton spectra of neurine hydrogenation at 45 Celsius and a pressure of 6.2 bar. Top spectrum is the untreated solution of neurine plus the catalyst.

1e. Determine the optimal polarization time to maximize the polarization of choline

The optimal polarization time is decided by how much hydrogenation has occurred in the reaction chamber. The polarization transfer requires transferring the spin state of parahydrogen added to the molecule before it can relax to the equilibrium state. This optimization has not been completed and is expected to be finish by 04/30/2012.

1f. Animal protocol approval (Months 1-3)
The animal protocol has been approved

Key Research Accomplishments

- Working parahydrogen induced polarization (PHIP) equipment
- Designed and implemented a stable magnetic field

- 100% hydrogenation of neurine to a choline analog in 4 seconds
- Derived the correct scalar coupling constants and wrote the software and r.f. transfer sequence for polarization

Reportable Outcome

Milestone – to achieve >10% polarization of the choline analog with near 100% conversion.

We have achieved the 100% conversion which allows us to minimize any toxicity effects of neurine and will soon check the polarization value.

Conclusion

We have completed and crossed the technical challenges in producing the PHIP equipment in order to finish *in vitro* and *in vivo* pilot studies

References

1 . M. Goldman, and H. Jóhannesson. *"Conversion of a proton pair para order into 13C polarization by rf irradiation, for use in MRI"* C. R. Physique 6 (2005).

Appendix
none